PB82-186321

# Biogeochemistry of Benzanthracene in an Enclosed Marine Ecosystem

Rhode Island Univ., Kingston

Prepared for

Environmental Research Lab.
Narragansett, RI

Mar 82

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| 1. REPORT NO. | 2. | 3. |
|---|---|---|
| EPA-600/J-80-387 | Journal Article | PB82-186321 |

| 4. TITLE AND SUBTITLE | 5. REPORT DATE |
|---|---|
| Biogeochemistry of Benzanthracene in an Enclosed Marine Ecosystem | March 1982 1980 |
| | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) Kenneth R. Hinga, Michael E. Q. Pilson, Richard F. Lee, John W. Farrington, Kjell Tjessem, and Alan C. Davis | 8. PERFORMING ORGANIZATION REPORT NO. ERLN-X24 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>University of Rhode Island<br>Graduate School of Oceanography<br>Marine Ecosystems Research Laboratory<br>Kingston, RI 02881 | 10. PROGRAM ELEMENT NO. ACUL1A/CCUL1A<br>11. CONTRACT/GRANT NO. CR807795 |
| 12. SPONSORING AGENCY NAME AND ADDRESS<br>Environmental Research Laboratory<br>South Ferry Road<br>Narragansett, RI 02882 | 13. TYPE OF REPORT AND PERIOD COVERED Journal Article<br>14. SPONSORING AGENCY CODE EPA/600/05 |

15. SUPPLEMENTARY NOTES
Environmental Science & Technology, Vol. 14, pp. 1136-1142 (1980)

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

REPRODUCED BY
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INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 2216

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS (This Report) | 21. NO. OF PAGES |
|---|---|---|
| Release to Public | Unclassified | 7 |
| | 20. SECURITY CLASS (This page) Unclassified | 22. PRICE |

EPA Form 2220-1 (9-73)

EPA-600/J-80-387
Journal Article

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# Biogeochemistry of Benzanthracene in an Enclosed Marine Ecosystem

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■ Carbon-14 labeled benz[*a*]anthracene was introduced into a large-scale marine microcosm containing an ecosystem functioning in a manner that was in many respects typical of the shallow coastal waters of the northeastern United States. The radiotracer label made it possible to follow the removal of benz[*a*]anthracene and labeled metabolites from the water column, their appearance and mixing into the sediments, and the production of $^{14}CO_2$ for 230 days. At the end of the experiment, 29% had been respired to $CO_2$, while the remaining extractable activity (43%) was evenly divided between parent compound and intermediate metabolic products.

The biogeochemistry of polycyclic aromatic hydrocarbons (PAHs) in coastal and estuarine ecosystems is important because of the deleterious effects some of these compounds may have on natural resources and human health (*1–3*). PAHs are released to the environment by a variety of processes such as oil spills, combustion of fossil fuels, forest and grass fires, and industrial effluents (*4–8*). Analyses of cores containing a historical record of near-shore marine and lake sediments (*9–13*) indicate increases in concentrations of PAHs by as much as an order of magnitude from ca. 1850 to the present. We can expect that fossil fuel combustion as currently practiced will continue to cause increasing levels of PAHs in the marine environment, especially if there is increased coal combustion in the next century (*4, 5*). Thus, it is important to understand the biogeochemistry of polycyclic aromatic hydrocarbons in coastal and estuarine areas in order to provide a basis for an evaluation of their long-term effects on these ecosystems.

The approach utilized in this experiment was to introduce a $^{14}C$-labeled PAH, benz[*a*]anthracene, into a large marine microcosm. The concentrations of labeled parent compound, some of its metabolites, and $^{14}CO_2$ generated from the breakdown of the labeled compounds were measured in various compartments of the ecosystem in the microcosm tank. Since these microcosms contain functioning marine ecosystems (*14–17*), the study was conducted under reasonably natural conditions, with natural populations, for a period of

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time approaching 1 year. It was possible to account for essentially all the labeled compound added to the system. Benz[*a*]anthracene was chosen because it occurs in many crude oils and refined products, is a major constituent of PAH in combustion products, and is on the Environmental Protection Agency's list of unambiguous pollutants (*6*, *8*).

*Experimental*

A large microcosm, one of 12 maintained by the Marine Ecosystems Research Laboratory (MERL) of the University of Rhode Island, was used for this study. Each microcosm is a fiberglass tank 1.8 m in diameter and 5.5 m in height, containing 13 $m^3$ of water and a 30-cm layer of sediment (*15*). The sediment in the tanks came from central Narragansett Bay and included a soft bottom community dominated by the polychaete *Mediomastis ambesita* and the bivalve *Nucula anulata*. During normal operation, approximately 120 L of water from adjacent Narragansett Bay (28–32 ‰ salinity) was pumped by a diaphragm pump into each tank four times daily, resulting in a washout rate of 3.7% per day. (This is a procedural improvement over the continuous flow regime noted in ref *15*. Flow rates were checked weekly.) The tanks were mixed for 2 h, four times a day, to simulate tidal currents and the turbulence regime in Narragansett Bay.

The MERL microcosms have, over several annual cycles, simulated adjacent Narragansett Bay with respect to biotic structure and chemical concentrations, processes, and rates (*14*–*17*). The enclosed ecosystems are large enough to allow many samples to be taken without noticeable depletion, and containment of the ecosystem makes it possible to identify the fate of nearly all the added material.

On May 29, 1978, approximately 200 $\mu$Ci of 12-[$^{14}$C]benz[*a*]anthracene (Amersham Corporation, 49 mCi/mmol) was introduced into a MERL tank within an oil–water mixture. This mixture was prepared by shaking 20 mL of no. 2 fuel oil with 17 L of bay water. After 30 min of settling, the bottom 15 L was transferred to another bottle, and ~0.93 mg of [$^{14}$C]benz[*a*]anthracene in 19 mL of acetone was added to the oil–water mixture. The bottle was shaken and the mixture introduced into the tank at a depth of 2 m during a mixing cycle. Absorption of benz[*a*]anthracene onto the surfaces of the containers used during the spike resulted in some loss prior to addition into the tank; therefore, the estimate of the amount introduced was obtained by extrapolating concentrations in the tank water back to the time of addition. An identical oil addition except for the [$^{14}$C]benz[*a*]anthracene–acetone mixture was made in another MERL tank. Gas chromatographic analysis (*18*) of the water in this tank 1 h after the addition of the oil–water mixture indicated that ~1 g of oil had been added.

**Sample Collection and Direct Counts.** All samples except cores were taken during a mixing cycle, to ensure that the water column was homogeneous during sampling. Four liters of water was siphoned into glass bottles and 20 mL of hexane was added; the mixture was stirred for 5 h and then allowed to sit overnight. After the hexane layer was drawn off with a pipet, 50 mL of chloroform was added, the mixture was stirred for 4 h and allowed to settle overnight, and the chloroform layer was removed. Aliquots (1–2 mL) of the hexane extract samples were placed in 10 mL of scintillation cocktail (Aquasol, New England Nuclear) and counted for $^{14}$C activity in a Beckman Model LS-3150T liquid scintillation counter at MERL. Duplicate samples were counted at Woods Hole Oceanographic Institution (WHOI) by adding appropriate aliquots of the extract to Aquasol scintillation cocktail and counting in a Beckman Model LS-100C liquid scintillation counter. Chloroform samples were also counted at WHOI by the same procedure. Counting efficiencies in all cases were 90% or better. MERL samples were prepared immediately after collection. WHOI hexane samples were stored at −15 °C, while chloroform samples were kept refrigerated.

Samples of particulate material were obtained by filtering 1 or 2 L of water through a 47-mm diameter Gelman A/E glass fiber filter. The filter was placed into 10 mL of cocktail and counted. Phytoplankton samples were obtained by a reverse flow concentration (*19*, *20*) of 2 L of water down to 3 mL through a 10-$\mu$m nylon screen. One milliliter of the reverse flow concentrate was placed directly in Aquasol and counted. Zooplankton samples from 350 L of water were obtained by taking a single vertical tow the height of the tank with a 30-cm diameter no. 20 net (80 $\mu$m mesh). The volume of the sample was measured and an aliquot (approximately 30%) was filtered through a glass fiber filter and counted. Samples were prepared immediately for counting or stored frozen until processed at WHOI.

Half-liter samples of water were transferred to a bottle fitted with two suspended wells and acidified to a pH of <2 with 5 mL of 0.2 M sulfuric acid. Evolved $CO_2$ was collected on two filters, one in each well, soaked with 0.2 mL of phenethylamine (New England Nuclear), while the sample was gently shaken. After 6 h the filters were replaced with fresh filters and the sample was extracted an additional 10–18 h. Both pairs of filter pads were then counted.

Cores were taken in 11-mm diameter polyethylene core liners and then immediately frozen while still in the liners.

**Separation of Intermediate Breakdown Products.** Procedures to separate and identify intermediate breakdown products (including products of metabolism and chemical and photooxidation) from the parent [$^{14}$C]benz[*a*]anthracene were conducted both at MERL and at WHOI. At MERL, aliquots of the hexane sample were taken to dryness using a nitrogen jet at room temperature and then made up to 0.2 to 0.4 mL with benzene. One aliquot of the benzene concentrate was counted; another was applied to silica gel thin-layer plates (Merck Co.) and run in a solvent system (benzene–hexane, 3:7 v/v) to separate polar metabolites from benz[*a*]anthracene. After we ran the sample on thin-layer plates, the benz[*a*]anthracene layer was identified by UV fluorescence of standard benz[*a*]anthracene. Three areas were marked, one corresponding to benz[*a*]anthracene (identified by the standard), one to a region containing highly polar metabolites (near the origin on the thin-layer plate), and the remaining middle region of the plate corresponding to hydroxybenz[*a*] anthracenes (9-hydroxybenz[*a*]anthracene and *cis*-5,6-dihydro-5,6-dihydroxybenz[*a*]anthracene standards provided by A. R. Patel through the National Cancer Institute, Carcinogenic Research Program) and some quinones run with this solvent system. The silica gel from each area was scraped from the plates, added to scintillation fluid, and counted. Aliquots of the zooplankton samples, phytoplankton (reverse flow) samples, and duplicate samples of total particulate material were each placed in a separatory funnel and extracted overnight with chloroform–methanol (*21*). The chloroform layer was removed, taken to dryness, and brought up to 0.2 or 0.4 mL with benzene. Aliquots of the benzene concentrate were counted and separated by thin-layer chromatography (TLC) as above.

Cores processed at MERL were extruded while frozen and sliced horizontally at 1-cm intervals. The 1-cm sections of the cores were placed in a round-bottomed flask with 5 mL of benzene and 5 mL of 0.5 N potassium hydroxide in methanol. Each sample was boiled with refluxing for 5 h. After cooling, the mixture was centrifuged to separate the sediment from the liquid. Aliquots of both the benzene and the methanol–water layers were counted. For selected cores, benzene and methanol–water aliquots were dried and used for thin-layer chromatography.

To determine the efficiency of the counting process, samples

were counted, a known activity of [$^{14}$C]toluene was added, and the samples were recounted. Efficiencies for most samples were over 95%, except for those of the sediment extracts, which were 55 to 95%.

Duplicate samples were processed at WHOI using different procedures. Hexane and chloroform extracts were evaporated to 2–10 mL, extracted three times with 2–5 mL of warm tetrahydrofuran (THF)–2-propranol (1:1), and then passed through a Sephadex LH-20 cleanup procedure. Both the chromatography and the extractions were carried out with only red light present, in order to minimize photooxidation of benz[*a*]anthracene metabolites. Sephadex LH-20 was allowed to swell overnight in 2-propanol, packed into a 58 cm × 2.54 cm glass column, and equilibrated for 12 h with a constant flow of 24 mL/min before a sample was loaded onto the column. A flow rate of 24 mL/min was found to be a good compromise between elution time and separation efficiency for this column. 2-Propanol as the mobile phase elutes aromatic hydrocarbons from Sephadex LH-20 by increasing ring number, while more polar compounds like quinones, alcohols, and acids are retained on the column. The elution time for benz[*a*]anthracene was established in 2-propanol, and all hydrocarbons up to benz[*a*]anthracene were eluted with 2-propanol. Benz[*a*]anthracene and the more polar metabolites were then eluted with THF at a flow rate of 80 mL/min. Substitution of THF for 2-propanol did not cause any problem as the gel beads swell the same in both solvents. The column was then reequilibrated with 2-propanol before introduction of the next sample. Standards used to establish LH-20 and high-pressure liquid chromatography elution conditions were benz[*a*]anthracene-*cis*-5,6-diol, 10,11-epoxybenz[*a*]anthracene-*trans*-8,9-diol (anti), 10,11-epoxybenz[*a*]anthracene-*trans*-8,9-diol (syn), and benz[*a*]anthracene provided by IIT Research Institute with support from the National Cancer Institute.

Particulate matter and no. 20 mesh net zooplankton samples at WHOI were extracted three times with 50 mL of warm THF–2-propanol (1:1) and three times with 50 mL of warm ethyl acetate, and the extracts were subjected to the cleanup procedure described above. Sediment samples were extracted three times with 50 mL of cold THF–2-propanol (1:1), three times with 50 mL of warm THF–2-propanol (1:1), and three times with 50 mL of warm ethyl acetate. The extracts were then evaporated to a small volume, centrifuged to remove small sediment particles not removed by decantation in previous steps, and subjected to cleanup on Bio-Beads SX-12 by eluting with acetone and toluene. Acetone as the mobile phase elutes the aromatics from Bio-Beads SX-12 by increasing ring number. The retention times for selected standards [including benz[*a*]anthracene, 9-hydroxybenz[*a*]anthracene, and 8,9-dihydrodiolbenz[*a*]anthracene] were measured in acetone, and the isolation and collection of benz[*a*]anthracene and metabolites in the sample extracts were based on these retention times. The flow rate was maintained at 30 mL/h. Elementary sulfur and other materials sticking to the gel bed were eluted with toluene, and the columns were reequilibrated with acetone before the next run. Elution with toluene was at a rate of 74 mL/min.

The hydrocarbon and metabolite isolates were analyzed on a DuPont LC-850 high-pressure liquid chromatograph (HPLC) equipped with a Zorbac ODS column and a filter photometer UV detector operating at 254 nm. Approximately 40 μL was loaded into a 50-μL loop. The elution of benz[*a*]anthracene and its metabolites was accomplished by running a gradient from 40 to 90% acetonitrile in water buffered to pH 4.0 with formic acid. The solvent gradient changed in a linear fashion at 2%/min. The flow rate was maintained at 2.0 mL/min and the temperature kept at 25 ± 1 °C. The elution times of the various groups of metabolites were established through the retention indexes of selected standard compounds. Only a few benz[*a*]anthracene standards are available, so the retention times of the quinone group were established by using benz[*a*]pyrenequinone standards. Various groups of peaks were collected from HPLC, and the activities were measured by scintillation counting as described above.

The background concentration of benz[*a*]anthracene originally present in the microcosm sediments was measured by quantitative gas chromatography–mass spectrometry as previously described (*18*, *22*).

**Table I. Extraction Efficiencies, during Preparation for TLC or HPLC, from Samples of Particulate Matter, and the Retention of Labeled Material during Evaporation of Hexane from the Hexane Extract of the Water**

| sample day | % recovery MERL | % recovery WHOI | sample day | % recovery MERL | % recovery WHOI |
|---|---|---|---|---|---|
| | Total Particulates | | | Zooplankton | |
| 1 | 82 | 75 | 1 | 63 | 20 |
| 2 | 56 | 70 | 3 | 53 | 24 |
| 3 | 75 | 33 | 5 | 37 | 22 |
| 4 | 60 | 87 | 7 | 56 | |
| 5 | 66 | 72 | 12 | 27 | 61 |
| 7 | 56 | 90 | 18 | 49 | 114 |
| 9 | 45 | | | | |
| | Phytoplankton | | | Hexane | |
| 1 | 60 | 80 | 2 | 89 | |
| 2 | 62 | 81 | 3 | 82 | |
| 3 | 60 | | 4 | 86 | |
| 4 | 74 | | 5 | 87 | |
| | | | 7 | 79 | |
| | | | 9 | 91 | |

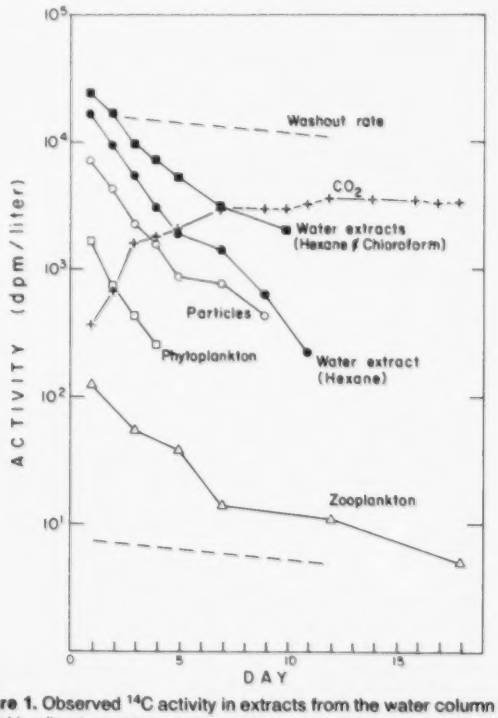


**Figure 1.** Observed $^{14}$C activity in extracts from the water column obtained by direct counting of various kinds of particulate materials during the first 18 days. The washout rate, shown by dashed lines, is the rate that $^{14}$C activity in the various samples would decrease as a result of water replacement in the tank

*Results*

The efficiency of the extraction techniques may be determined for total particulate, phytoplankton, and zooplankton samples by comparing direct counts (of subsamples in scintillation fluid without extraction) to the counts of the extracts from duplicates of samples to be used for TLC or HPLC. The extractions from total particulates and phytoplankton were generally 60–80% efficient, while the extractions from zooplankton were less efficient, about 20–60% (Table I). A check of the methanol–water phase and of fractions of the solid residue from methanol–chloroform extractions of the particles, phytoplankton, and zooplankton showed that the remainder of the activity could be accounted for in those portions. Hexane samples after drying were found to have 85% of their original activity (Table I). There was good agreement for direct count determinations on duplicate hexane samples when the results for the same day obtained by two different laboratories were compared.

**Water Column.** One day after the spike all samples from the water column had significant amounts of $^{14}C$ activity (Figure 1). The $^{14}C$ activity of the total (hexane + chloroform) water extract decreased during the first few days with a half-life of about 50 h, while that of the particulates, phytoplankton, and zooplankton decreased with a half-life of about 35 h for the first 5 days and then more slowly until the activity in the samples became too low for counting. The decrease in $^{14}C$ activity in these samples was much greater than would be expected from the washout of the tank. During the same interval the $^{14}C$ activity present as dissolved carbon dioxide ($\Sigma CO_2$) increased. Measurements of the mass of particulates, phytoplankton, and zooplankton were made for the same time period in other MERL microcosms; comparison with these data suggests that the specific activities of the three fractions ($^{14}C$ activity/gram dry weight) were approximately equal.

Thin-layer chromatography and HPLC analysis of water extracts, particulates, phytoplankton, and zooplankton showed that the fraction of total $^{14}C$ activity in these extracts attributable to [$^{14}C$]benz[*a*]anthracene decreased with time (Figures 2 and 3). The TLC analysis showed that [$^{14}C$]-benz[*a*]anthracene decreased from 70% of the $^{14}C$ activity on day 1 to 20% by day 9, while polar metabolites increased in percentage during the same interval. HPLC analysis of both hexane– and chloroform–water extracts showed a similar decrease in [$^{14}C$]benz[*a*]anthracene. Although this trend is clear, it should be noted that the extraction procedures did not recover all the $^{14}C$ activity measured in the samples by direct counts (Table I). The unextracted $^{14}C$ activity is likely to be in highly polar metabolites. The relative amount of [$^{14}C$]benz[*a*]anthracene shown in Figure 2 is based on $^{14}C$ in the lipid fraction. The THF extractions and cleanup procedures select primarily benz[*a*]anthracene and its alcohol and quinone metabolites. The HPLC analysis of the cleaned hexane– and chloroform–water extracts missed 30–40% of the $^{14}C$ activity present in the extracts. This again indicates that some of the $^{14}C$ activity from the labeled benz[*a*]anthracene had been incorporated into relatively polar organic compounds as a result of the metabolism of benz[*a*]anthracene.

Chloroform extracted labeled compounds remaining in the water after hexane extraction (Figure 1). After day 3 the hexane extracted less than half of the total materials extractable by hexane followed by chloroform. HPLC analyses of the chloroform extracts also showed that the hexane procedure missed a substantial portion of the benz[*a*]anthracene.

The $^{14}C$ activity present in the water as $\Sigma CO_2$ reached a maximum on day 12 (Figure 4). Thereafter, the concentration of $\Sigma^{14}CO_2$ decreased, indicating that the production of $^{14}CO_2$ became less than the sum of losses from washout, escape of gaseous $^{14}CO_2$ to the atmosphere, and biological fixation. From day 63 to day 77, the water flow to the tank was stopped. During this period of batch operation there was no washout of $\Sigma^{14}CO_2$, and the concentration of $^{14}CO_2$ in the tank increased. This increase clearly demonstrated that $^{14}CO_2$ was

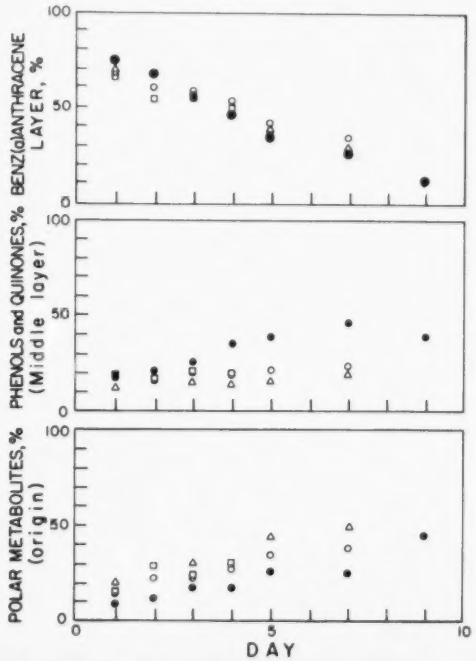


**Figure 2.** Distribution of radiolabeled benz[*a*]anthracene and metabolites determined by thin-layer chromatography of hexane extracts of the water (●), and on particulate samples (○), zooplankton (△), and phytoplankton (□)

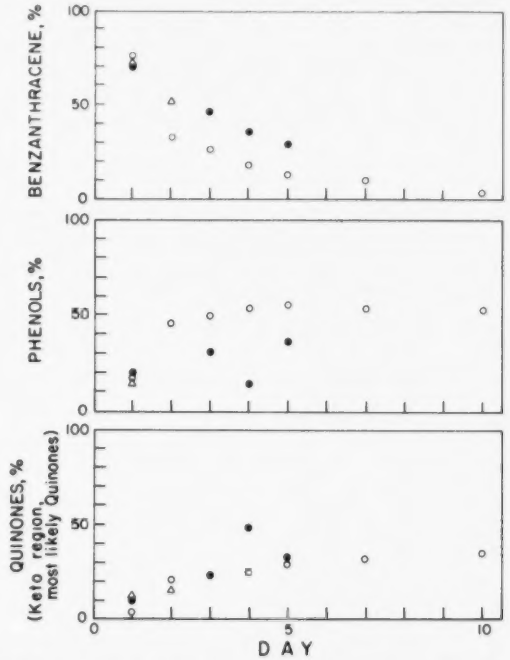


**Figure 3.** Distribution of radiolabeled benz[*a*]anthracene, phenols, and quinones determined by high-pressure liquid chromatography of hexane (●) and chloroform (○) extracts from the whole water samples and extracts from filtered samples of particulate material (△).

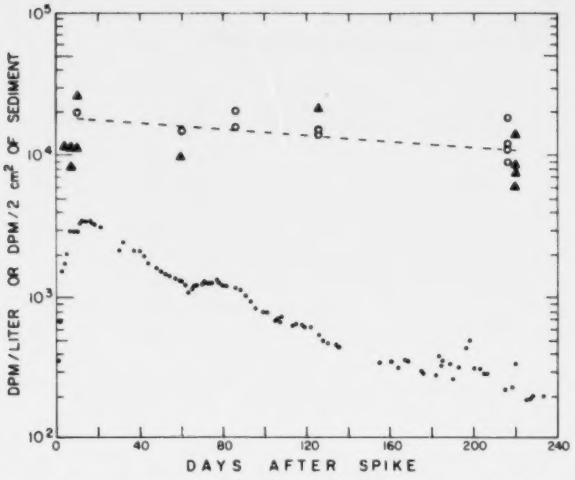


**Figure 4.** Concentration of $^{14}CO_2$ in water (●) and total $^{14}C$ activity extracted from sediment cores at MERL (O) or at WHOI (▲). The dashed line is a linear regression of data from the measured cores from day 10 to 220. On this scale the curvature associated with plotting in a semilog graph is not visible

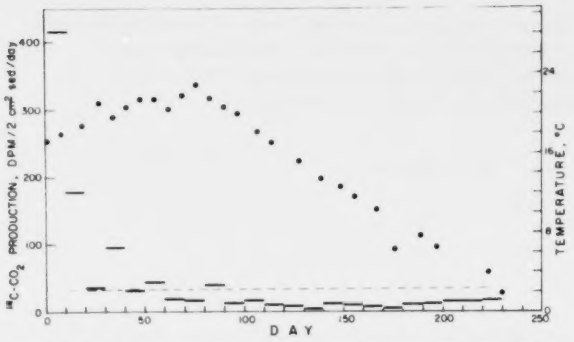


**Figure 5.** Solid lines are 10-day averages of the production of $^{14}CO_2$ calculated from $\Sigma^{14}CO_2$ concentrations in water. Dashed line is production of $^{14}CO_2$ determined by the decrease in $^{14}C$ activity in measured cores, using the linear regression from Figure 4. Closed circles are temperature of the tank water during the experiment

still being produced, though at a rate much slower than during the first few days of the experiment.

The washout rate from the tank is known, and the sum of the rates of $\Sigma^{14}CO_2$ loss to the atmosphere and biological fixation in a MERL tank is 1% of the total $\Sigma^{14}CO_2$ each day (*23*). Biological fixation as used here refers to labeled carbon incorporated into the biota and not regenerated by respiration to $CO_2$ during the course of the experiment. Thus, it is possible to calculate the $^{14}CO_2$ production for each day of the experiment using the equation:

$$P = (D_2 - D_1) + \text{washout} + (0.010D_1)$$

where $P$ is the $^{14}CO_2$ production, $D_1$ is the $\Sigma^{14}CO_2$ in the tank on any day, and $D_2$ is the $\Sigma^{14}CO_2$ in the tank on the following day. Washout is calculated from flow records, and the final term is a calculation of $\Sigma^{14}CO_2$ lost to the atmosphere and biological fixation. The rate of $\Sigma^{14}CO_2$ loss to biological fixation is probably variable but certainly small compared to the $\Sigma^{14}CO_2$ loss to the atmosphere, so the combined loss appears to be relatively constant over time. The $^{14}CO_2$ production (plotted in 10-day intervals to smooth the data, Figure 5) decreased during the first 40–60 days of the experiment and remained at a low but measurable level until the experiment was terminated at day 230.

**Sediment.** Labeled benz[*a*]anthracene and its labeled metabolites, excepting $CO_2$, disappeared from the water column after a few days. The major portion of the $^{14}C$ activity was found in the sediment with a lesser portion appearing as $\Sigma CO_2$ (Figure 4). The total activity in quadruplicate cores taken the same day showed variability by a factor of 2, but the data sets from the two laboratories were not distinguishable (Figure 4). Activities in cores are reported as disintegrations per minute under each 2 $cm^2$. The geometry of the tanks results in each 2 $cm^2$ of the sediment lying under exactly 1 L of water. Most of the $^{14}C$ activity was initially associated with the top centimeter of sediment (Figure 6). As time progressed the labeled compounds were found deeper, a result expected from studies of particle transport (*24, 25*) and benthic ecology (*26, 27*). There was substantial variability or patchiness in the distribution of these compounds both vertically and horizontally. Once incorporated into the sediment, the labeled compounds survived for at least 220 days and were lost slowly. The TLC analyses of extracts from a few selected cores showed that the fraction of total activity present as benz[*a*]anthracene was about 40–60% in surface sections and about 5–15% in sections 4 to 5 cm down the core (Figure 6). Table II lists the fractions of the $^{14}C$ activity that were present as $CO_2$, benz[*a*]anthracene, or intermediate metabolites on various days.

**Mass Balance.** An attempt was made to identify the fate of all the $^{14}C$ activity that was introduced into the tank. At any one time the total $^{14}C$ activity must equal the sum of that in the water, in the sediment (or on the walls of the tank), washed out of the tank, and lost as a gas to the atmosphere. If the total obtained by adding all results from measurements of $^{14}C$ activity is close to the amount added to the tank, a reasonable assurance is provided that the sampling and analytical procedures used were not severely deficient. For calculation of the mass balance the following equation was used:

$$\text{total} = [^{14}C]\text{sediment} + [^{14}C]\text{water} + \Sigma[^{14}C]\text{washout} + \Sigma[^{14}C]\text{atmosphere}$$

The spike $^{14}C$ activity (31 000 dpm/L) was obtained by ex-

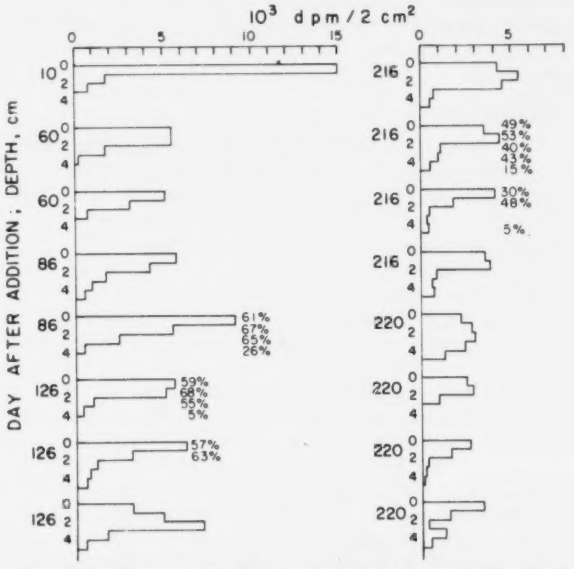


**Figure 6.** Distribution of $^{14}C$ activity with depth in sediment cores taken at intervals through the experiment from day 10 to 220. The numbers given in some cases to the right of the profiles at 1-cm intervals show the percentage of the $^{14}C$ activity in each interval which ran with benz[*a*]anthracene during thin-layer chromatography

**Table II. Percentage of the Total $^{14}C$ Activity Added to a MERL Tank as [$^{14}C$]Benz[*a*]anthracene and Recovered as Parent Compound, $^{14}CO_2$, and Intermediate Metabolic Products**

| day | benz[*a*]anthracene [a] | $CO_2$ [b] | intermediate products [a] | total accounted for [c] |
|---|---|---|---|---|
| 1 | | 1 | | |
| 86 | 34 | 26 | 23 | 94 |
| 126 | 29 | 28 | 23 | 91 |
| 216 | 21 | 29 | 22 | 83 |

[a] Includes, after day 86, data from sediments only, as water column values were then negligible. [b] All $CO_2$ was in the water column. Values include estimate of $CO_2$ washed out and $CO_2$ lost to atmosphere by air–water exchange. [c] Includes the amount of [$^{14}C$]benz[*a*]anthracene and intermediate metabolic products, estimated at 11% of total initial activity, washed out of the tank during approximately the first 20 days of the experiment.

**Table III. Mass Balance of $^{14}C$ Activity Added as [$^{14}C$]Benz[*a*]anthracene to One MERL Tank [a]**

| day | found in water column | calcd cumulative loss through washout and gas exchange | found in sediment (range) | total accounted for |
|---|---|---|---|---|
| 4 | 29 | 8 | 38 | 75 |
| 7 | 20 | 10 | 32 (26–37) | 62 |
| 10 | 15 | 12 | 62 (37–86) | 89 |
| 60 | 4 | 31 | 40 (31–48) | 75 |
| 86 | 4 | 33 | 58 (50–65) | 95 |
| 126 | 2 | 37 | 52 (45–65) | 91 |
| 216 | 1 | 40 | 39 (27–56) | 80 |
| 220 | 1 | 40 | 28 (19–44) | 69 |
| | | | mean | 80 |

[a] All values expressed as a percent of the initial estimated value of 31 000 dpm/L.

trapolating the hexane + chloroform extract line from Figure 1 to the time of the spike. The [$^{14}C$]water is the summation of $^{14}C$ activity in the hexane + chloroform extracts plus the $\Sigma^{14}CO_2$. The washout for each day is the $^{14}C$ activity in the water times the portion of the water removed by washout. The $\Sigma$[$^{14}C$]washout is the summation of the calculated daily washouts. The $^{14}CO_2$ loss to the atmosphere (and to biological fixation) was calculated daily in the same manner as was done to estimate the $^{14}CO_2$ production. Benz[*a*]anthracene lost to the atmosphere is thought to be negligible (*28*). The results of these calculations (Table III) indicate that the mean total estimated recovery was 80% of the amount added, and there was no evident trend in this value with time. The 5 to 40% of the $^{14}C$ activity that was apparently missed during the various samplings was probably in the form of benz[*a*]anthracene or its metabolites not extracted from the sediments or, in the early days of the experiment, from the water column. No absolute calibration of the extraction efficiencies from either medium was attained in these experiments. However, the mean extraction efficiency from water column particulate material was 63% (Table I). Some activity may also have been adsorbed on the tank walls, although in experiments with other hydrocarbons this has not been a significant factor (*18*). At present we cannot evaluate these possible sources of error.

*Discussion*

Both the microbial and benthic animal populations of the microcosm originated from a coastal site near populated areas; therefore, they must have had prior exposure to benz[*a*]anthracene (*8, 10*). Quantitative gas chromatography–mass spectrometry measurements of benz[*a*]anthracene in control MERL sediments gave concentrations of 200–300 ng/g dry weight. Most of the benz[*a*]anthracene and other PAHs in the sediment likely originate from a combination of low-level chronic inputs of petroleum hydrocarbons and fossil fuel combustion products (*8, 10*). Thus, these systems have probably received anthropogenically enhanced inputs of benz[*a*]anthracene for more than 100 years, in addition to lower level inputs from natural fires since the estuary was formed (*7, 8, 10*). If all the approximately 0.93 mg of benz[*a*]anthracene added during the experiment described above had been incorporated into the sediment, the increase in concentration would have been 27 ng/cm$^3$ in the top 1 cm of sediment, about one-sixth of the existing concentration. Thus, the added benz[*a*]anthracene did not greatly change the prior concentration.

The total $^{14}C$ activity, including both the parent benz[*a*]anthracene and labeled breakdown products, was removed from the water with a half-life of about 52 h, while the [$^{14}C$]benz[*a*]anthracene itself had a half-life of 24 h, a behavior similar to that of benz[*a*]anthracene in another microcosm experiment (*29*). Based on knowledge of the general microbial and animal metabolism of aromatic hydrocarbons and photochemical reaction of benz[*a*]anthracene, it seems likely that a combination of microbial and photochemical processes gave rise to the labeled products which were found in the water and in the sediments. After a few days, animal metabolism and excretion might also have produced some metabolites (*30–34*). The presence of phenol and quinone metabolites in the sediment and in the water during the early stages of the experiment demonstrates that the metabolism of benz[*a*]anthracene to $CO_2$ was not rate limited by the initial conversion to quinones or phenols. Once they were formed they survived long enough to be detected in substantial quantities, up to 23% of the initial total $^{14}C$ activity.

Benz[*a*]anthracene, having a low water solubility and low rate of metabolism in the water column, became associated with sediments either through direct adsorption on the bottom after turbulent mixing of water to the sediment–water interface or by adsorption onto or incorporation into suspended particles and subsequent deposition. Once incorporated into surface sediments, the benz[*a*]anthracene and its metabolites were mixed deeper into the sediments by benthic animal activity. Both the removal and mixing processes took place on a time scale which was faster than the conversion rate of benz[*a*]anthracene and extractable metabolites to nonextractable metabolites and $CO_2$, as the total $^{14}C$ activity in the sediment did not decrease rapidly.

Because of the rapid transfer of the benz[*a*]anthracene to the sediment, it is not possible to specify whether the initial high rate of metabolite production was carried out in the water column on suspended particulates or in the sediment. However, studies of [$^{14}C$]benz[*a*]anthracene in bottle experiments (*35*) using water and sediments from both Narragansett Bay and the MERL microcosms indicated that no $^{14}CO_2$ production occurred in water, while a measurable $^{14}CO_2$ production took place in sediments. The greater percentage of $^{14}C$-labeled metabolites in the top centimeter of sediment than in the 1–2-cm layer indicates that the metabolism of benz[*a*]anthracene occurred more rapidly in the top layer. For other coastal sediments it has been shown that microbial degradation of petroleum hydrocarbons is more rapid near the surface than in lower layers (*36*). In the MERL sediments this may be due in part to the reducing conditions below 1 cm, while the surface sediments are oxidized (*37*). The increased $^{14}C$ activity found in polar metabolites, relative to that in the parent compound, deeper in the core (4–5 cm layer) provides some

insight into diagenetic processes and suggests further studies. This observation could perhaps be explained by assuming a greater diffusive mobility of the polar compounds, compared to benz[*a*]anthracene, or by the action of benthic animals ingesting and partially metabolizing the benz[*a*]anthracene and then excreting the metabolites at depth.

The variability of the depth distributions for quadruplicate cores on days 216 and 220 illustrates that, despite the passage of 200 days since the introduction of the benz[*a*]anthracene to the system, the combination of metabolic processes and biological and physical mixing of sediments had not resulted in a uniform distribution of either parent compound or extractable metabolites in the upper 4 to 5 cm of sediment. This is consistent with the view that in benthic ecosystems of this type there is a dynamic interaction of fluid and particle movements brought about by different benthic animals with varying feeding strategies and burrowing activities, resulting in local patchiness of chemical distributions.

The data do not allow an unequivocal calculation of how long it would take for the remaining $^{14}C$ activity to be converted to $^{14}CO_2$. The $^{14}CO_2$ production followed neither a linear nor an exponential loss rate (Figure 5). We do not know the mechanisms that control the rate of benz[*a*]anthracene breakdown, so we have no basis to choose a method of calculation. If the $^{14}CO_2$ production rate were to remain the same as during the last 100 days of the experiment (Figure 5), all the labeled benz[*a*]anthracene would be converted to $^{14}CO_2$ within 3.5 years after the spike. Since the temperature of the sediments would be greater in the summer, it may be more reasonable to use the decrease in $^{14}C$ activity in sediment cores from day 10 to day 216, which includes warmer temperatures, to predict 2.0 years for the complete mineralization of the benz[*a*]anthracene. Assuming an exponential loss rate and depending on the interval chosen, half-lives of 1.2 to 3 years may be calculated. The occurrence, however, of benz[*a*]anthracene at some depth in natural sediments suggests that a fraction of this compound and perhaps some of its metabolites may persist indefinitely.

Because so many of the better known chemical functions in the MERL ecosystems follow those in the adjacent bay, both in magnitude and timing, it seems reasonable to suggest that the behavior of benz[*a*]anthracene in the microcosm would be similar to that in Narragansett Bay and similar coastal bodies of water. Nevertheless, with the present state of our knowledge, it would be risky to extrapolate from these data to the situation in other environments. Temperature, water–sediment exchange processes, rates of bioturbation, physical characteristics of the sediment, and other effects may help control the benz[*a*]anthracene degradation rate. If the addition had been in the winter and then progressed to the warmer months of summer, the benz[*a*]anthracene loss through microbial and animal metabolism might have been different. During the winter when there is less rapid bioturbation, the benz[*a*]anthracene may remain for a longer period in the surface sediments. In regions of rapid sedimentation, or in areas subjected to destruction of the biota by an oil spill, benz[*a*]anthracene buried in the sediment may remain in anoxic layers and be protected to some extent from microbial attack. The persistence of metabolites and reaction products raises important questions with regard to assessing the environmental risk associated with levels of aromatic hydrocarbons in coastal ecosystems. Many of the metabolites and reaction products of aromatic hydrocarbons are thought to be mutagenic and/or carcinogenic (*31*). Measurements of the loss of aromatic hydrocarbons after a spill do not provide a measure of the metabolites which may persist. This is especially true for the heavier molecular weight phenanthrenes and naphthalenes and compounds such as benz[*a*]anthracene and benzo[*a*]pyrene, which persist longer than the lower molecular weight benzenes and naphthalenes (*22*). The persistence of these compounds suggests the presence of a hazard. However, further measurements and experiments are needed to determine to what extent these compounds are available for uptake by biota and subsequent long-term effects.

*Acknowledgments*

We thank Dr. Patrick Gearing, Dr. Juanita Gearing, Dr. James Quinn, Dr. Candace Oviatt, and Mr. David Johnson for helpful discussion and cooperation. Drs. J. and P. Gearing also provided the analysis of total oil, and made several editorial suggestions. We also thank the entire MERL staff for their helpfulness.

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*Received for review January 11, 1980. Accepted April 14, 1980. This work was supported by Environmental Protection Agency Grant R 80607202. This paper is Woods Hole Oceanographic Institution Contribution No. 4513.*